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Expression of HIV *gag* and *env* B-cell Epitopes on the Surface of HBV Core Particles and Analysis of the Immune Responses Generated to Those Epitopes

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An investigation into the presence of T-cell epitopes within the hepatitis B virus (HBV) has led to the identification of a potential universal carrier molecule in the form of the hepatitis B core antigen (HBcAg) particle. The HBc particle, which exhibits unique immunological characteristics, can be engineered to carry multiple, heterologous epitopes from other pathogens that may ultimately serve as vaccine candidates. We have selected human immunodeficiency virus (HIV) *gag* and *env* sequences as candidate B-cell epitopes to be positioned on the surface of HBc particles and expressed as fused proteins. The DNA constructs were prepared in a manner that would not alter the inherent T-cell functions and particulate nature of the HBc particles.

Unique Immunological Characteristics of HBc Particles

The use of hepatitis B particulate antigen as a T-cell carrier protein is not unique to HBc. The hepatitis B surface antigen (HBsAg) has been utilized as a T-cell carrier protein for heterologous B-cell epitopes. A comparison of the humoral response generated to HBcAg versus that observed with HBsAg suggests that the core antigen of HBV, rather than the surface antigen, represents a more potent immunogen and hence a better T-cell carrier. The development of antibody to the HBsAg is not achieved by all individuals infected with HBV; however, virtually all of those infected with the virus develop antibody to HBc. A comparison of HBc with HBs in seven congenic murine strains demonstrated that HBc could elicit high antibody titers in all strains tested, whereas HBs was reactive in only five of the seven, with the generation of low-to-moderate antibody titers (Milich and McLachlan 1986).

The ability of HBcAg to function as a T-cell-independent antigen has also been dem-

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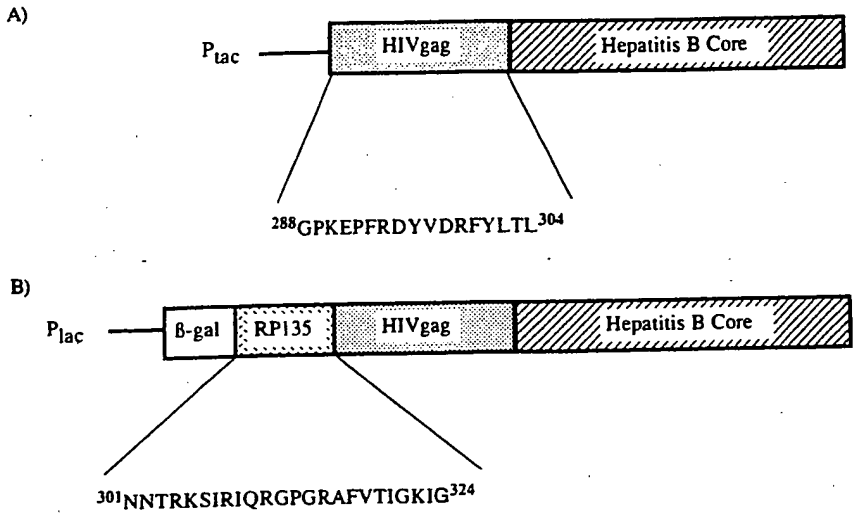

Figure 1
(*A*) An HIV *gag* sequence (288–304), inserted upstream of the HBc-coding sequence cloned in vector pKK223-3, was expressed as a fusion protein in *E. coli*. The synthetic DNA oligomer, complementary to the selected amino acid sequence, was inserted at *Eco*RI site in the multicloning site of vector. (*B*) An HIV *env* sequence (RP135) was inserted upstream of the HIV *gag*/HBc clone in plasmid pBS at the *Pst*I-*Bam*HI insertion site of the vector.

onstrated with the particulate form of HBcAg. Immunization of a euthymic strain of mouse and its athymic counterpart with particulate HBc resulted in antibody production in both strains, demonstrating that HBcAg could function as a T-cell-independent antigen (athymic), as well as a T-cell-dependent antigen (euthymic) (Milich and McLachlan 1986). This unique immunological characteristic was not due to the particulate nature of HBc alone in that HBs particles were unable to elicit antibody by a T-cell-independent pathway. Additionally, it had been demonstrated that attachment of a hapten (DNP) to HBc through chemical coupling resulted in the same T-cell independence exhibited by HBc (Milich et al. 1988). All of these immunological features exhibited by particulate HBc demonstrate its uniqueness as a T-cell carrier molecule.

DISCUSSION

Expression of a T/B-cell Epitope from the HIV *gag* Region on the Surface of HBc Particles

To determine if HBc particles can act as a carrier for a foreign B-cell epitope, we selected the HIV *gag* region of HIV-1 for investigation. Our intent was to select peptide sequences that provide T- and B-cell functions and present them on the surface of HBc particles to take advantage of the unique cellular mechanisms that have been defined for HBc. A peptide sequence (288–304), predicted by standard algorithms to contain both a T- and B-cell epitope within a limited amino acid region (17-mer) (Coates et al. 1987), was inserted upstream of the HBc coding sequence (AM6) (Moriarty et al. 1981) in the *Escherichia coli* expression vector pKK223-3 (Pharmacia). A DNA oligomer representing the nucleotide complement of the 288–304 amino acid sequence (Fig. 1A) was inserted at the *Eco*RI site of the vector. This particular peptide was weakly immunogenic

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Determining the A
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Antibody used to capture particles:	*Labeled antibody to probe for antig*
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Table 1
Determining the Antigenicity of the Purified pKK/HIV*gag*/HBc Particles and Confirming the Location of the HIV Epitope on the Surface of HBc

Antibody used to capture particles:	–	–	–	–	*Anti-288–304*
Labeled antibody used to probe for antigen:	*Anti-HBc*	*Anti-288–304*	*Anti-p24*	*Anti-HIV lysate*	*Anti-HBc*
Purified HBc	1.618	.019	n.d.	.011	.012
Purified HIV*gag*/HBc	1.626	1.920	1.852	.771	1.827

Numbers represent OD values recorded at 490 nm. The same concentration of antigen was used for both preparations (100 ng). The antibodies used to probe for antigen were either rabbit polyclonal antibodies generated to peptides and whole proteins or mouse monoclonal antibodies specific for HBc. n.d. indicates not determined.

on its own; it was able to raise antibody in both rabbits (after three immunizations) and mice (BALB/c), demonstrating that a T-cell site was contained within the 288–304 sequence that was capable of eliciting antibody in the animals tested. In addition, it should be noted that a human serum sample positive for HIV-1 was capable of reacting with this peptide in ELISA. Also, antibodies raised in rabbits to both a recombinant p24 preparation (Centocor) and an HIV lysate were able to react with the 288–304 peptide, suggesting that it may contain a native B-cell site.

A DNA construct (pKKHIV*gag*/HBc) was used to express the hybrid protein in *E. coli*, and the fused product was detected by Western blot analysis with antibody specific for denatured HBc and an anti-p24 rabbit antibody. A protein with the predicted size of 23,000 M_r (the HIV 17-mer plus linker sequences, six precore amino acids, and the full HBc sequence [21,000 M_r]) was demonstrated. The material was purified to homogeneity by means of gel filtration, ion-exchange chromatography, and hydroxyapatite binding. The material obtained after the purification procedure was analyzed by ELISA and determined to be particulate (reactive with anti-HBc), and the HIV *gag* epitope was positioned on the surface of the particles (anti-288–304 could capture the particles) (Table 1). Purified HIV *gag*/HBc particles were reactive with anti-HBc, anti-288–304, anti-p24, and anti-HIV lysate. The particulate nature of the material was subsequently confirmed by electron microscopy, where the hybrid particles were found to be identical in shape, although slightly larger in size, than native HBc particles (31 nm vs. 27 nm).

Immune Response in Mice Generated to the Purified HIV*gag*/HBc Particles

The purified HIV *gag*/HBc particles were used to immunize mice, previously identified as nonresponders to peptide 288–304 (B10 and B10.S). The peptide was presented both linked to keyhole limpet hemocyanin (KLH) and on the surface of HBc particles (HIV *gag*/HBc particles) to compare the immune response generated to the attached epitope as a function of the carrier molecule (Table 2). The antibody responses generated were evaluated by reactivity with peptide 288–304 (antipeptide) and a recombinant p24 protein (anti-*gag*). A primary immunization was administered at day 0, followed by a boost at day 30. The animals were bled at days 24 and 44. Peptide 288–304, administered alone, is nonreactive in both strains of mice even after a boost. The delivery of peptide 288–304 linked to KLH resulted in antipeptide titers at day 24 in both strains; however, there was no anti-*gag* response at the same time point. The presentation of peptide on the surface of HBc particles resulted in a response similar to the antipeptide response

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Table 2
Antibody Titers Generated in B10 Mice Strains to HIV*gag* Epitopes

Immunogen	Antipeptide		Anti-gag	
	B10	B10.S	B10	B10.S
288–304				
primary	0	0	0	0
boost	0	0	0	0
288–304-KLH				
primary	1,280	320	0	0
boost	80,000	1,280	20,000	320
288–304/HBc				
primary	1,280	1,280	1,280	1,280
boost	>80,000	5,000	>80,000	5,000

The concentrations of immunogen used were 100 μg peptide, 20 μg of peptide linked to KLH, and 10 μg of HIV*gag*/HBc particles. The values represent reciprocal titers. Animals were bled 24 days after a single immunization (primary) and 14 days after a boost at day 30 (boost).

recorded for the peptide-KLH preparation at day 24; however, an anti-*gag* response was generated after only a single immunization, which increased significantly after the boost. The presentation of the HIV *gag* epitope on the surface of HBc particles was more immunogenic than the peptide linked to KLH (higher antibody titers) and elicited a stronger and more rapid anti-native response. Further immunological analysis in higher animals should confirm the effectiveness of HBc as a carrier for foreign epitopes.

Insertion of an HIV *env* Sequence Upstream of the HBc and HIV*gag*/HBc Sequences

The T-cell help provided by HBc for the carried B-cell epitope is nonspecific with regard to the foreign sequence. In an attempt to provide specific T-cell help to a carried B-cell epitope, an HIV envelope sequence identified as a neutralizing epitope (RP135) (Rusche et al. 1988) was inserted upstream of the HIV *gag*/HBc fusion protein. We selected a *gag* T-cell sequence over those predicted for the envelope region in an effort to determine if the HIV *gag* region influences the immune response generated to the HIV envelope in a manner similar to that demonstrated with HBV (Milich et al. 1987). The internal viral protein of HBV (HBc) regulates the immune response generated to the HBV envelope proteins (HBs). Insertion of a B-cell epitope from the HIV envelope upstream of an HIV *gag* T-cell site, and positioning both on the surface of HBc particles, has been accomplished. A DNA oligomer complementary to the RP135 sequence was inserted at the *Pst*I-*Bam*HI site of plasmid pBS (Stratagene) in which the HIV *gag*/HBc-coding sequence was downstream (Fig. 1B). The final product is a fusion protein consisting of five β-galactosidase amino acids, linker sequences, the HIV *gag* 17-mer, six precore residues, and the entire HBc sequence. The predicted size for the fusion protein is 26,000 M_r. Western blot analysis revealed a protein species migrating at the predicted molecular weight for the fusion protein recognized by antibody specific for HBc and the RP135 determinant. ELISA confirmed the presence of particles (reactive with anti-HBc) and the surface location of the RP135 envelope sequence (anti-RP135 can capture the hybrid particles). These particles are currently being purified for immunological analysis.

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SUMMARY

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SUMMARY

The ability of HBc to carry epitopes (HIV *gag* and *env*) on its surface, while maintaining particle formation, has been demonstrated. The presentation of B-cell epitopes in this manner versus that observed when coupled to KLH generated higher antipeptide and anti-*gag* antibodies in two strains of mice that were nonresponders to the peptide alone (B10 and B10.S). The ability of the HIV *gag* epitopes to demonstrate T-cell independence when presented on the surface of HBc particles remains to be elucidated. The ability of HIV *gag* to influence the immune response generated to the envelope of HIV will be determined with the hybrid particles expressed with the DNA construct β-gal/RP135/HIV *gag*/HBc.

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